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Flow Cytometry: First Principles, Second Edition. Alice Longobardi Givan

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Flow Cytometry

First Principles

Second Edition

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Alice Longobardi Givan

The Herbert C. Englert Cell Analysis Laboratory
of the Norris Cotton Cancer Center
and Department of Physiology
Dartmouth Medical School
Lebanon, New Hampshire

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*The First Edition of this book was dedicated to my parents,
Violet Litwin Longobardi and Vincent Longobardi, Jr.,
with gratitude for the example they set,
with pride in their achievements,
and with love.*

*The Second Edition is dedicated to
Curt, Ben, and Becky
(not only because I promised them that their turn would come).*

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Preface

Although flow cytometry is simply a technique that is useful in certain fields of scientific endeavor, there is, at the same time, something special about it. Few other techniques involve specialists from so many different backgrounds. Anyone working with flow systems for any length of time will realize that computer buffs, electronics experts, mathematicians, optical and fluidics engineers, and organic chemists rub shoulders with biologists, physicians, and surgeons around the flow cytometer bench.

And it is not just a casual rubbing of shoulders, in passing, so to speak. Many of the specialists involved in flow cytometry might, if asked, call themselves *flow cytometrists* because the second aspect of flow cytometry that distinguishes it from many other techniques is that flow cytometry has itself become a “field.” Indeed, it is a field of endeavor and of expertise that has captured the imaginations of many people. As a result, there exists a spirit of camaraderie; flow cytometry societies, groups, meetings, networks, websites, journals, courses, and books abound.

A third aspect of flow cytometry (known sometimes simply with the acronym for fluorescence-activated cell sorter, *FACS*, or even more familiarly as just *flow*) that distinguishes it from many other techniques is the way in which its wide and increasing usefulness has continued to surprise even those who consider themselves experts. What began as a clever technique for looking at a very limited range of problems is now being used in universities, in hospitals, within industry, at marine stations, on submersible buoys, and on board ships; plans have existed for use on board space ships as well. The applications of flow cytometry have proliferated (and continue to proliferate) rapidly both in the direction of theoretical science, with

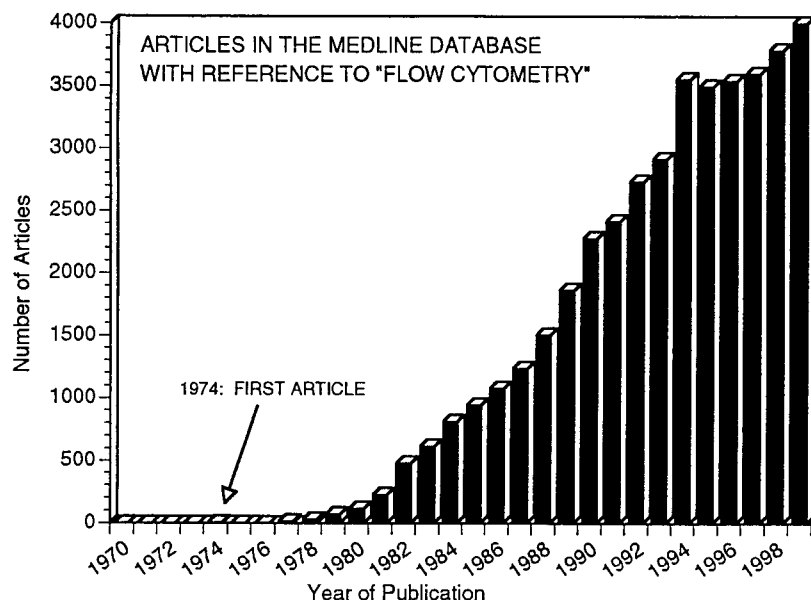


Fig. 1. Increasing reference to "flow cytometry" in the medical literature over the past three decades. The development of flow cytometers antedates the use of the term itself.

botany, molecular biology, embryology, biochemistry, marine ecology, genetics, microbiology, and immunology, for example, all represented; and in the direction of clinical diagnosis and medical practice, with hematology, bacteriology, pathology, oncology, obstetrics, and surgery involved. We are, at present, living through what appears to be a rapid phase in flow cytometry's growth curve (see Fig. 1).

Because flow cytometry is an unusual field, bringing together people with differing scientific backgrounds at meetings, on editorial boards, in hospital wards, on advisory panels, and at laboratory benches and reaching increasing numbers of workers in new and unpredicted areas of endeavor, there is, as a result, a need to provide both recent and potential entrants into this diverse community with a common basis of knowledge—so that we can all understand the vocabulary, the assumptions, the strengths, and the weaknesses of the technology involved. I have for many years taught new and future users of flow cytometers. My teaching attempts to present enough technical background to enable students, scientists, technologists, and

clinicians to read the literature critically, to evaluate the benefits of the technique realistically, and, if tempted, to design effective protocols and interpret the results. I try to describe the theory of flow cytometry in a way that also provides a firm (and accurate) foundation for those few who will go on to study the technique in greater depth. Details of protocols are avoided, but my teaching attempts to give enough information about applications to provide concrete examples of general concepts and to allow some appreciation of the range of practical goals that the instruments are able to achieve. With some expansion (but with little change in style or objectives), my classroom and workshop teaching is the basis for this book.

Notes on the Second Edition: This new edition, while similar to the first edition in style and scope, has been modified in many ways. The arrangement of some material has been altered to present, in my opinion, a more coherent pedagogical sequence, reflecting my changing thoughts on teaching. While all chapters have been re-written to a significant extent, there are also some major expansions reflecting the progress of the field. In particular, I have included more detail about the cell as it passes through the laser beam; the laser/fluorochrome chapter has been expanded to include recently developed fluorochromes and multilaser options; a new chapter has been added on cytoplasmic staining; a discussion of apoptosis has been added to the chapter on DNA; the section on sorting has been expanded to a full chapter and includes high-speed sorting and alternative sorting methods as well as traditional technology; the clinical and research chapters have been updated and expanded considerably; the chapter on general references includes many of the recent excellent books in the field; and the chapter on the future of flow cytometry is now a subjective glimpse into the new decade from the vantage point of the year 2000.

Acknowledgments

(First Edition)

Realizing how much I have learned and continue to learn from others, I hesitate to single out a few names for particular mention. However, with the disclaimer that any list of people to whom I am indebted is not meant to be and, indeed, could never be complete, I must thank here the following friends, mentors, and colleagues who have had a very direct impact on the writing of this book: George Proud, for having had enough insight into the importance of a flow cytometer for transplantation surgery to want to have one; Ivan Johnston and Ross Taylor, for hospitality (and the Harker Bequest Fund, the Northern Counties Kidney Research Fund, and the Newcastle Health Authority, for financial assistance) within the Department of Surgery at Newcastle University; Brian Shenton, for introducing me to the field of flow cytometry and to the joys and hazards of clinical research; Mike White, for bailing me out (often figuratively and once or twice literally) on so many occasions; Paul Dunnigan, for teaching me about lasers (and also about fuses, relays, and loose wires); Ian Brotherick, for the animal amplification figure, and both he and Alison Mitcheson for good humor in the lab beyond all reasonable expectation; Terry Godley, for being an extremely good and (more importantly) a very communicative flow cytometry service engineer; Ray Joyce, for considerable assistance with the design and drawing of many of the diagrams; the scientists and clinicians at Newcastle University and Durham University (many acknowledged in the figure legends), for providing me with a continuous source of interesting and well-prepared cell material on which to practice my flow technique; Paul Guyre, for giving me a supportive and remark-

ably enjoyable re-introduction to science in the New World; Daryll Green, for giving me the benefit of his expertise in both physics and flow cytometry by reading and commenting about the chapters on instrumentation and information; Brian Crawford, for being a literate and encouraging editor in the face of my rank inexperience; the late Robert L. Conner, for providing me with that critical first of my still-continuing scientific apprenticeships; Curt Givan, for his unfailing loyalty and for his skill in reading the entire manuscript with two eyes—one eye that of an old-fashioned grammarian who abhor dangling participles and the other that of a modern scientist who knows nothing about flow cytometry; and Ben Givan, for the two drawings in Figure 8.1 [Fig. 10.1 in the Second Edition], Becky Givan, for organizational magic when it was very badly needed, and both kids for *lots* of encouragement and some pretty funny suggestions for a title.

A.L.G., 1991, Newcastle upon Tyne.

Acknowledgments

(Second Edition)

I continue to be immensely grateful to the people whom I acknowledged in the First Edition of FCFP; their contributions are still current and form the foundation for this new edition. I have, however, during preparation of the new edition, received considerable additional help. During the 9 years since the publication of the first edition of this book, changes have taken place in my life as well as in the field of flow cytometry. Now working at Dartmouth Medical School, I have learned much about clinical cytometry, flow analysis, and computers from Marc Langweiler; Marc taught me the acronym “RTFM,” but has been restrained at using it in response to my naïve questions and I am grateful. In addition, Marc was a particular help to me with suggestions for Chapter 10. Gary Ward, the flow sorting supervisor in the Englert Cell Analysis Laboratory at Dartmouth, has, by his competence, made my sorting skills rusty, but he has allowed me to pretend that I run “his” sorter when I need a dramatic photograph, and I am grateful for his good humor. I am also grateful to Ken Orndorff, who supervises the imaging service in the Englert Lab; he has been admirably tolerant of flow cytometrists (including me) who have slowly begun to realize that a dot on a dot plot may not be enough and that they need to know what their cells look like. In addition, I want to thank the many users of the instruments in the Englert Lab for their ability to provide me with continuing challenges.

First Colette Bean and then Luna Han at Wiley inherited editorial supervision of this book from Brian Crawford and I owe them considerable thanks for their persistence and patience. At Dartmouth, I am grateful for secretarial assistance from Mary Durand and artistic

help from Joan Thomson. Dean Gonzalez at Wiley, with artistry and stamina, is responsible for the great improvement in figure clarity between the two editions of this book. I thank him.

During these last 9 years, I have had the opportunity to expand my acquaintance with members of the greater flow community. Among many from whom I have learned much, I would like especially to mention a few: Louis Kametsky shared with me his lecture notes for a wonderful keynote address he gave at Dartmouth on the history of flow cytometry, and I am grateful to him. Howard Shapiro has been a source of limitless cytometric information cloaked seductively in outrageous humor; I thank him for his knowledge, for his humor, and for his generosity. Paul Robinson has made many contributions to flow cytometry, but I want particularly to mention his organization of the Purdue University flow network; I am grateful to him for the information, the moral support, and the distance friendships that the network has given me. Over the years, I have had the privilege of teaching workshops and courses with a group of people who are devoted not just to flow technology (although they are talented flow cytometrists) but also to education of students in the correct use of the technique. Indeed, I also detect their devotion to the use of flow cytometry as a platform for teaching a rigorous approach to science in general. In thanks for all I have learned from these workshops, I wish, particularly, to mention here Bruce Bagwell, Ben Hunsberger, James Jett, Kathy Muirhead, Carleton Stewart, Joe Trotter, and Paul Wallace.

And—as ever—Curt Givan came through with unflappable proof-reading skills and many cups of tea.

A.L.G., 2000, Lebanon, New Hampshire.

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